

appearance, smell and taste<sup>2</sup>. On identifying a suitable partner — female, young (preferably virgin) and of the same species — the suitor serenades her with a love song produced by the vibration of one of his wings<sup>6</sup>. The behaviour of the female seems more passive, or at least too subtle for human voyeurs to detect easily; but it is she who ultimately decides whether to allow the male to initiate copulation.

These gender-specific differences in courtship behaviour are largely determined by a male-specific transcription factor called Fru<sup>M</sup>, which is expressed in 1–2% of the more than 100,000 neurons in the fly nervous system<sup>7</sup>. Neurons expressing this factor (Fru<sup>M</sup> neurons) include sensory cells for odours, tastes, sounds and sights; central neurons in the brain; and motor neurons that control wing and leg movements<sup>7,8</sup>. This hints that Fru<sup>M</sup> neurons form an interconnected circuit<sup>7,8</sup>. Although Fru<sup>M</sup> is expressed and essential only in males, female flies contain similar classes of neurons that are required for reproductive behaviours such as egg-laying<sup>9</sup>.

Surprisingly, initial work<sup>7</sup> reported no dramatic sexual dimorphisms of these neural pathways — apart from differences in Fru<sup>M</sup> expression — that could account for the distinct male and female courtship behaviours. More recent investigations<sup>10–12</sup>, however, found that small subpopulations of these neurons have gender-specific properties. The three new studies<sup>3–5</sup> take a closer look at Fru<sup>M</sup> neurons in males and their counterparts in females to address two fundamental questions: do they really form an interconnected circuit; and how widespread are sexual dimorphisms? To scrutinize these neural pathways, each team used a distinct approach — a worthy reminder of the power of fly genetics for dissecting brain structure and function.

Yu *et al.*<sup>4</sup> devised an ‘intersectional’ genetic strategy to express reporter proteins in small, consistent subsets of Fru<sup>M</sup> neurons in individual brains, which allowed them to visualize the projections of these neurons with greater clarity than before. Cachero *et al.*<sup>5</sup> used a clonal marking method, in which subpopulations of Fru<sup>M</sup> neurons that derive from the same neural stem cell were labelled.

The authors<sup>4,5</sup> applied each approach exhaustively, to identify more than 100 distinct groups of Fru<sup>M</sup> neurons throughout the nervous system. Moreover, they reconstructed *in silico* a comprehensive (although hypothetical) ‘wiring diagram’ of the Fru<sup>M</sup> neural circuits, by digitally reconstructing the morphology of these neurons’ projections — dendrites and axons, which receive and transmit neural signals, respectively — and by integrating the mapped neurons into a common reference brain (Fig. 1). This allowed them to predict the flow of information from one set of neurons to another, as well as to locate brain regions essential for integrating the diverse types of sensory message that pass between males and females during courtship.

In contrast to these ‘global’ analyses, Ruta *et al.*<sup>3</sup> focused on a single olfactory pathway that is responsive to *cis*-vacenyl acetate (cVA) — a male-specific pheromone that promotes sexual receptivity in females but inhibits courtship in males<sup>13</sup>. To visualize the neural components of this pathway, the authors expressed in all Fru<sup>M</sup> neurons a photoactivatable reporter protein, PA-GFP, that can be converted from a low to a high fluorescence state with a pulse of high-energy light<sup>12</sup>. They then activated PA-GFP in precisely the brain area that is innervated by the axons of cVA-responsive olfactory sensory neurons. As this region also contains the dendrites of neurons to which these sensory cells connect, PA-GFP was simultaneously activated in this second population of neurons, and its diffusion revealed the neurons’ axonal projections in higher brain centres.

Through this elegant, iterative photolabelling approach, Ruta *et al.* could thus move stepwise through the brain to the output neurons that are likely to link directly with motor pathways. Although the circuit they define corresponds to just a small piece of the wiring diagram defined in the larger-scale anatomical studies<sup>4,5</sup>, Ruta *et al.*<sup>3</sup> go one crucial step further by confirming that these cells are functionally connected. They accomplish this by recording cVA-evoked activity in each of the identified neurons — an impressive achievement deep in the tiny fly brain — and by demonstrating that this activity depends on the presence of intact circuit components upstream.

What do these findings reveal about the neural control of courtship? First, they offer a draft roadmap of a circuit underlying a complex animal behaviour. Although relatively simple neural circuits for reflexes (such as gill withdrawal in the marine slug *Aplysia*<sup>14</sup> or the escape response in the fruitfly<sup>15</sup>) have been delineated, the Fru<sup>M</sup> circuit is the most sophisticated to be mapped so thoroughly, sometimes down to single-neuron resolution. As the male courts his target female, these neurons must integrate diverse sensory information. Yet, as Ruta and co-workers show for the cVA response pathway, the circuit can be surprisingly shallow, with as few as four neurons potentially linking sensory input to motor output. The techniques and tools these studies<sup>3–5</sup> introduce also make it feasible to test the functional contributions of individual subpopulations of Fru<sup>M</sup> neurons to these behaviours in males and females.

In addition, these studies identify an unexpected number of new sexual dimorphisms in Fru<sup>M</sup> neurons, including several cases in which certain groups of these cells are present only in the male or the female brain. They also detect hundreds of putatively distinct connections between the axons and dendrites of Fru<sup>M</sup> neurons that are common to both sexes (Fig. 1). Although these anatomical (and physiological) observations do not establish a causal relationship between dimorphic wiring and behaviour, they indicate that widely distributed, although



## 50 Years Ago

*Serengeti Shall Not Die* by Dr. Bernhard and Michael Grzimek — This is the book of the film. Despite the many fine illustrations, the book cannot compete with the film in showing the space of the Serengeti National Park and the beauty of its animals in motion ... It is a true adventure story. After the success of their first film “No Room for Wild Animals”, the Grzimeks offered part of its revenues for purchase of land to increase the Serengeti National Park, but were persuaded instead to study the animal populations there; that study entailed learning to fly, and that entailed getting permission from their wives.

From *Nature* 3 December 1960

## 100 Years Ago

We geologists who were privileged to take part in the journey to Spitsbergen before the meeting of the Geological Congress in Stockholm had good reason to count ourselves fortunate ... Not many hours after sinking Bear Island in the southward ... we began to meet ice-floe; which soon thickened, so that we had to slow down and eventually to turn southward and westward for more open water. Again and again during the day was this experience repeated, a chilly ice-blink always paling the hazy sky to the north and east as we threaded our zigzag course amid the floes, on which inquisitive seals shifted uneasily, doubtful whether to regard us as dangerous or not ... Soon, very gently, the haze thinned away; the northern sun shimmered again over the smooth olive sea, burnishing the floes into silver; and then, gradually, an exquisite panorama of peaks and glaciers was unveiled in front of us ... and we knew that this was Spits-bergen, and worthy of its name.

From *Nature* 1 December 1910